REGENERATION OF TISSUE COMPOSED OF PARTS OF TWO SPECIES.

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BORN's experiments in grafting together tadpoles of different species of frogs have demonstrated that each part, whether large or small, retains the characteristics of the species to which it belongs. During the development of an animal, formed by the union of parts of two species, the tissues do not influence each other, but each develops its own specific peculiarities.

Joest has shown that when parts of two different species of earthworms are grafted together each part retains its specific characters. He has further shown that if, after grafting, a portion of one of the parts is cut off, the new part that is regenerated is like the part from which it immediately arises, and is not influenced by the part belonging to the other species, even when the latter is very large, and the former (that from which the new part arises) is very small.

Many experiments have been made with plants in which different species have been grafted together, and the subsequent growth of the two parts studied. Vöchting, who has given a detailed account of these experiments and has made others himself, has shown that in general no influence of a specific character is transmitted from one part to the other, although in certain cases[1] the parts do have some influence on each other.

The following experiments were made, not so much to determine whether the tissues of one component of a graft influence the kind of regeneration of the other, since this point seemed fairly settled by Joest and by Harrison, but I hoped to find out if new tissue, made up of cells derived from parts of two species, showed any mixing of the specific characters of the two species.

[1] Particularly in those cases where annual and biennial varieties are grafted together.

It seemed possible, at least, that new tissue, composed of cells derived from two species, might show the influence of its dual origin.

Harrison[1] has shown that the tails of young tadpoles may be interchanged even when two species are used, and that later the ectoderm of the body of the larger component grows out over the base of the grafted tail, slipping over the region where the tail has been grafted on, as shown in Fig. 1. If two species be used, and then, after the tail has grown to the stage shown in this figure, the tail be cut off just distal to the point of union, as shown in Fig. 2 by the vertical line, there will be present at the exposed end two kinds of tissue — the ectoderm, which is the same as that covering the body of the tadpole, and the inner tissue, composed of muscles, connective tissue, pig-

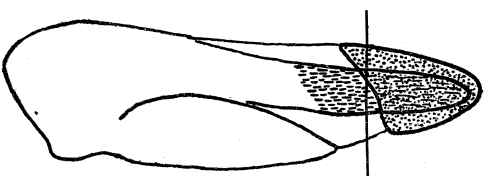

FIG. 1. (After Harrison.)

ment cells, notochord and nerve cord, that belong to the grafted tail. Under these circumstances the new tail that regenerates will be made up of parts of two species. Harrison carried out an experiment of this sort. He writes[2] in regard to the result: "The tail of a larva of *R. virescens* was replaced by the tail of a larva of *R. palustris*, in the manner described above. Forty-eight hours later, at which time the sketch was made (see Fig. 1), the tail was amputated. The epidermis from the virescens body had then pushed out considerably over the root of the tail, so that in cutting, almost all of the grafted epidermis (stippled in the figure) was removed. But a considerable portion of the underlying organs of the transplanted piece (shaded in the figure) remained, and it was from this component that regeneration took place in all the tissues, with the exception of part of the epidermis. The newly grown tail was

[1] Harrison, R. G., "The Growth and Regeneration of the Tail of the Frog Larva," *Roux's Archiv.* Vol. vii, 1898. [2] Page 473. Case 13.

of normal form, and, as far as could be observed, it had the characteristics of the species of the grafted stump (palustris) and not those of the body (virescens). This was seen in the character of the pigmentation, and especially in the absence of the large black blotches along the side of the tail, which are found constantly in the regenerated appendages of *R. virescens*. In spite of the insignificant size of the grafted stump, as compared with the whole body, and in spite of the fact that the nourishment conveyed to the growing appendage is brought there in blood, which is largely derived from the body, the tissues maintain their specific characters.[1]"

By using two species in which there is a marked difference in the pigmentation of the ectoderm and also some distinctive difference in the color of the pigment cells in the mesoderm, I hoped to be able to determine more definitely the character of the new part, and further, by observing the tissues of the two species, where they are regenerating side by side, to see if they mutually influence each other. The problem is somewhat different from the one Harrison examined, since I was less concerned with the influence of the major component on the new regenerating part than with the possibility of a mutual influence of the new cells on each other. Harrison has shown, with some degree of probability, that the former influence is not shown in the new part, but the latter problem is not specially considered.

I have found it possible to graft together two such differently pigmented tadpoles as *Rana (temporaria) sylvatica* and *R. palustris*. The former breeds earlier, but the development will be retarded several weeks if the dishes in which the tadpoles are placed be put on ice in an ice chest. It is better to let the tadpoles develop as far as the stage when the tail-knob is just about to appear, since at this stage they withstand better

[1] "I had hoped to obtain more definite evidence concerning the influences which regulate regeneration, from experiments carried out along these lines. But, owing to unfortunate circumstances, most of the larvae of this series died. Besides, all regenerated tails deviate somewhat from the normal type, especially as regards pigmentation, which fact would bring in a considerable element of uncertainty, and in the tail I have not been able to find any other characters which could with safety be considered diagnostic of either species."

the effect of the ice-cold water. If segmenting eggs or the early gastrula stages be put on ice they are killed after several days, although the latter stages withstand the cold longer than the former. The young tadpoles do not seem to be in the least injured, and may even slowly continue to develop, but at so slow a rate that after three weeks the tail had grown only a very little. Since in this locality the eggs of *R. palustris* can be obtained in great abundance for a period of at least two weeks, I have had plenty of material of both species.

The tadpoles were operated upon at the time when they had reached the age shown in Harrison's Fig. 2. They were still in the jelly membranes. Muscular movements of the body had scarcely begun at this time. The tadpoles were held in place by small pieces of aluminium wire. Silver wire used by Born and by Harrison would probably be better, since it is heavier.

The young tadpole of *R. sylvatica* is very black, the color being due to the deeply pigmented ectoderm and to some extent to pigment in the mesoderm. The young tadpole of *R. palustris* is much lighter in color. The ectoderm contains a yellowish pigment, and the pigment cells of the mesoderm are lighter in color than those of the other species. After grafting together parts of these two species, the difference in color of the two parts is so marked that it can be easily seen with the naked eye. Under the microscope one can tell readily whether an individual cell in the ectoderm belongs to the one or to the other species. In later stages, when the ectoderm has become clearer, the two kinds of cells can no longer be distinguished without a microscope. The core of the tail of *R. sylvatica* is much darker than that of *R. palustris*, and the line of union of the two can be seen with the unaided eye.

As the tadpole grows larger, it will be found that the ectoderm of the smaller component grows less rapidly than the rest of the tail, and as a result the ectoderm of the larger component extends over the base of the grafted tail, as Harrison has stated (Figs. 2 and 3). The tadpoles were allowed to grow for about ten days, or somewhat longer,[1] and then the tail was cut off in various ways.

[1] It would have been better to have cut the tail off sooner, since the difference in the ectoderm of the two species is less marked in later stages.

Experiment I. — The tail of *R. palustris* had been grafted upon the body of *R. sylvatica*. The tadpole appeared at the time of the second operation, as shown in Fig. 2 *A* (April 25). The dark ectoderm of the major component — *R. sylvatica* — had grown out over the base of the tail of the smaller com-

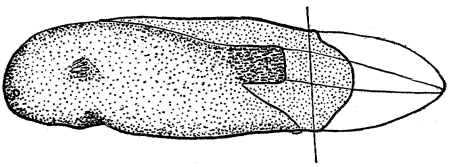

FIG. 2 *A*.

ponent (*R. palustris*). The region of union of the inner tissue can be seen where the dark and the light parts meet. The tail was then cut off, as shown by the vertical line in the figure. In consequence, there was left exposed at the cut end of the tail

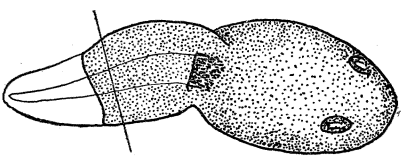

FIG. 2 *B*.

the inner tissues derived from *R. palustris*, and the outer from *R. sylvatica*. A new tail began to regenerate, and during all of its subsequent development the new tail was made up of ectoderm exactly like that of the major component, and of inner

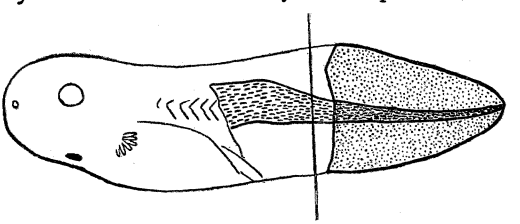

FIG. 3.

tissue whose pigment cells resembled those of the minor component. In other words, both inner and outer tissues regenerated their kind and showed no commingling of characters.

Experiment II. — In this experiment the major component was *R. palustris* and the minor *R. sylvatica*. After the new

tail had reached the stage shown in Fig. 3, it was cut off as indicated by the vertical line. There was left exposed at the cut end the light-colored ectoderm of the major component and the inner tissues of the minor component. The new tail that developed had light ectoderm on the surface and a dark interior. Each part regenerated its specific tissue and was uninfluenced by the developing tissue of the other species.

Two points present themselves for consideration. If the tail of an ordinary tadpole be cut off and subsequently develop, does the regenerated tail show the specific characters of the normal tail or is it different? I have examined the regenerated tails of both species and find that both the ectoderm and the mesodermal pigment cells are like those of a normal tail. It is, however, not very uncommon, both in regenerated tails of normal tadpoles and also in grafted tadpoles, to find the mesodermal pigment cells imperfectly developed, and in such cases the specific character of the cells is not obvious; but in all cases in which the pigment cells are well developed, the specific character is readily seen, especially in the cells lying along the central part of the tail. It should be stated, however, that I have occasionally found isolated cells whose character was doubtful, but the large majority of cells are unquestionably like those of the tissue from which the new tail arises. The second question is whether the ectoderm forms new cells over the new part, or does the old ectoderm simply extend out over the new part? There is the appearance in the regenerating tail of the formation of a new ectoderm at the tip of the new tail, where the cells are more crowded together and smaller than over the base of the tail. It is not improbable that in addition to this new ectoderm the old ectoderm extends also over a part of the new tail.

Experiment III. — In several cases the tail was cut off obliquely, in much the same way as in Harrison's experiment. Owing to the difference in pigmentation of the two kinds of ectoderm, I could follow the subsequent history of each and determine whether, along their line of contact, and in the region where new cells are developing, the specific characters of the cells are altered.

As shown in Fig. 4, the tail of a tadpole, in which the major component is *R. palustris* and the minor *R. sylvatica*, was cut off obliquely, leaving a small amount of the dark ectoderm of *R. sylvatica* on the upper side. The inner cells at the cut edge all belonged to *R. sylvatica*. When the new tail developed, it showed along its upper part the dark ectoderm of *R. sylvatica*, that had developed from the small piece left at the time of the

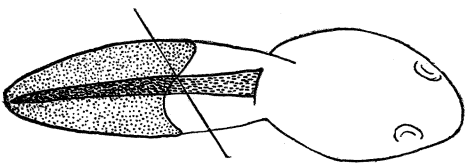

FIG. 4.

operation. The area covered by the dark ectoderm was greater than that left after the tail was cut off, but it cannot be stated how much of this increase is due to the cells becoming flatter and how much to new cells formed at the free edge.

In another similar experiment, in which, however, the major component was the dark species, *R. sylvatica*, and the minor the paler species, *R. palustris*, the tail was cut off (April 27), as shown in Fig. 5. A large area of light ectoderm was left on the dorsal surface of the tail, and only a small amount of the

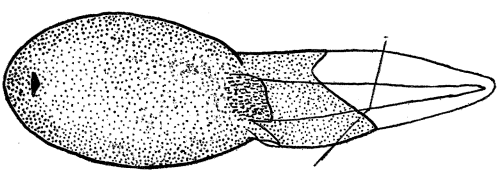

FIG. 5.

black ectoderm came to the edge of the lower part. On May 10, when the new tail was fairly well developed, it was found to have its upper surface covered by light-colored ectoderm, and its lower by dark ectoderm, while the interior mesodermal pigment cells were like those of *R. palustris*. Each tissue had regenerated its like, and the light ectoderm of the minor component showed no influence of the other, dark ectoderm, even along the line of contact where new cells were developing.

In addition to these experiments I have records of four others similar to Experiment I (Fig. 2, *A*, *B*); three others similar to Experiment II (Fig. 3); and four others in which the tail was cut off obliquely, leaving both kinds of ectoderm at the cut edge. In all cases the specific character of the new tissue was like that of the old tissue from which it arose.

At first the difference in the ectoderm of the two species is very marked, but as the tadpoles get older the ectoderm seems to flatten and become more transparent, so that in these tadpoles it is difficult to distinguish between the two kinds of ectoderm. But if the tadpoles are examined every day one can detect differences in the two kinds of ectoderm for a longer period than could be done by casual observations alone. Wherever the ectoderm has not spread out, particularly at the tip of the tail, the dark pigmented cells of *R. sylvatica* and the yellowish cells of *R. palustris* can be readily detected. The pigment cells in the mesoderm assume their characteristic arrangement during the older stages, and as the ectoderm becomes more transparent, the cells can be easily seen in the living tadpoles. The tadpoles were all kept under the same condition, so that the effect of light on the pigment cells would be approximately the same in all experiments.

Unfortunately the differences in pigmentation are the only specific characters that can be made out readily in these tadpoles, but I think there can be little doubt that if the cells retain their characteristic pigmentation they also retain their other peculiarities.

We may conclude with some degree of probability that during regeneration in a region where the cells have been derived from two different species, each kind of new cell retains the character of the cells from which it is derived, and the specific characters of the cells of one species are not transmitted to the cells of the other species, although the developing cells in the new tissue may be in actual contact.